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LeuT-Desipramine Structure Suggests How Antidepressants Inhibit Human Neurotransmitter Transporters

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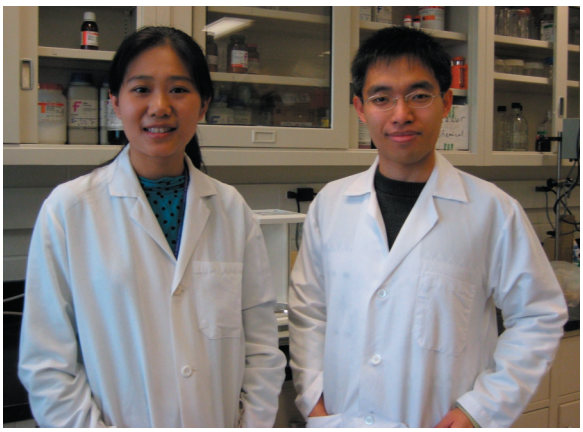
Tricyclic antidepressants exert their pharmacological effect – inhibiting the reuptake of serotonin, norepinephrine and dopamine – by directly blocking neurotransmitter transporters in the presynaptic membrane. We determined the crystal structure at 2.9 Å of the bacterial leucine transporter (LeuT), a homolog of the human transporters, in complex with leucine and the antidepressant desipramine. Desipramine binds at the inner end of the extracellular cavity of the transporter and this binding site is separated from the leucine-binding site. Mutagenesis experiments on human proteins indicate that both the desipramine-binding site and its inhibition mechanism are probably conserved in the human neurotransmitter transporters

Na^+/Cl^--dependent neurotransmitter transporters for serotonin (SERT), norepinephrine (NET), and dopamine (DAT) in the presynaptic plasma membrane terminate neuronal signal transmission in the central nervous system through a reuptake mechanism. These three systems have been shown to modulate mood, emotion, sleep, and appetite. Depression, the most prevalent psychiatric disorder, is directly associated with perturbation of serotonergic neurotransmission, and drugs have been used successfully for the treatment of depression. One class of drugs, tricyclic antidepressants (TCAs) such as desipramine, binds to serotonin and norepinephrine transporters and blocks their transport activity. However, their binding site in the transporter proteins had not been identified.

To investigate the molecular basis of TCA binding to LeuT, we co-crystallized LeuT with desipramine and solved the complex structure to 2.9 Å resolution. X-ray diffraction data were collected at NSLS beamline X29. The diffraction data was initially refined against the TCA-free LeuT structure. The final LeuT-desipramine complex model had an R_{free} 21.9% and an R_{work} of 20%. The bound desipramine molecule sits at the inner end of the extracellular cavity in LeuT (Figure 1). Importantly, the desipramine molecule is separated from the substrate leucine by the extracellular gate of the transporter, which consists of residues R30, Y108, F253, and D404. Thus, the desipramine-binding site and the leucine-binding site are non-overlapping. On the extracellular side, desipramine is held in place by the turn of the EL4 helix hairpin, and nitrogen N2 atom the tail of desipramine forms a salt bridge with D401. This crystal structure of LeuT-desipramine complex immediately suggests a mechanism for inhibition of substrate transport by the TCA molecule. Desipramine directly binds to the extracellular gate of the transporter and locks the gate by inducing formation of a salt bridge between R30 of TM1 and D404 of TM10. The formation of this salt bridge prevents tilting of TM1, which is believed to be required for substrate release to the cytosol. Thus, no substrate transport can occur.



Authors (from left) Juan Zhen and Zheng Zhou

We tested the relevance of this desipramine-binding site identified in the bacterial protein to neu-

rotransmitter reuptake inhibition by mutating key residues at the presumed TCA-binding site in the human neurotransmitter transporters SERT and DAT, followed by measuring their transport inhibition by desipramine in human embryonic kidney cells. We succeeded in generating gain-of-function mutants in terms of desipramine binding for both DAT and SERT proteins by mutating their key residues to those found in the sequence of NET. Such gain-of-function mutagenesis data clearly demonstrate that desipramine binds to the same site in both DAT and SERT as it does in LeuT and inhibits transport activity in the same manner.

Thus, in the human Na^+/Cl^--dependent neurotransmitter transporters SERT, NET, and DAT it is likely that tricyclic antidepressants also bind between the extracellular gate and EL4 hairpin, thereby inhibiting neurotransmitter reuptake at the synapse. In combination with homology modeling and molecular docking, the identification of this drug-binding site will allow studies on the interactions of SERT- and NET-specific inhibitors with these transporters and may aid in the structure-based design of more effective neurotransmitter reuptake inhibitors as antidepressants.

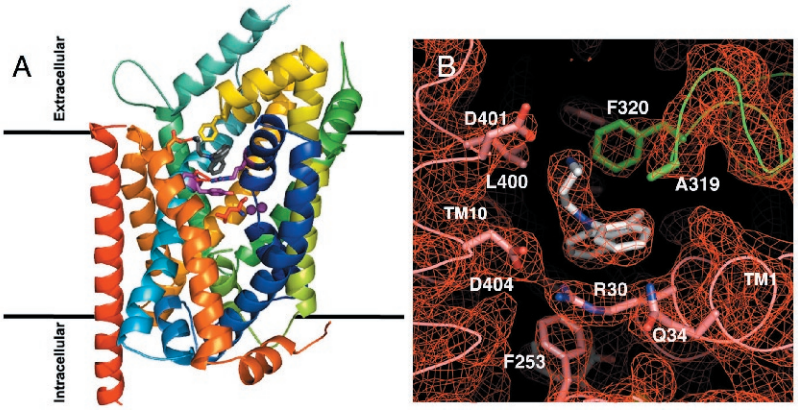


Figure 1. *Structure of the LeuT-desipramine complex. (A). Over structure of the LeuT-desipramine complex showing the position of the bound desipramine, viewed from within the membrane plane. (B). $2F_o$-F_c map contoured at 2 σ showing the desipramine binding site in LeuT, viewed from within the membrane plane. Residues R30, Y108, F253 and D404 form the extracellular gate, which separates the leucine substrate from the bound desipramine.*